Reprogramming of mouse fibroblasts into cardiomyocyte-like cells in vitro.

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Public Summary:
Structural cells in the heart called cardiac fibroblasts, which represent 50% of the cells in the mammalian heart, can be directly reprogrammed to beating heart muscle-like cells in mice that have experienced a heart attack. We delivered three genes that normally guide embryonic development (abbreviated as GMT) directly into the damaged region of the mouse heart. Within a month, non-beating cells that normally form scar tissue transformed into beating heart cells. Within three months, the hearts were beating even stronger and pumping more blood. This process is more difficult to achieve in a petri dish, but would be useful for testing new drugs for personalized medicine or modeling cardiac disease in a dish. Here, we describe a detailed, step-by-step protocol for reprogramming cardiac fibroblasts in a dish using a retrovirus to introduce the GMT genes into the cells. Developing standardized protocols will help research laboratories master the procedure and develop the technology further. Initial evidence of reprogramming can be observed as early as three days after the procedures begin. The reprogrammed cells begin to beat in the dish after approximately 2 months.

Scientific Abstract:
Cardiac fibroblasts can be reprogrammed to cardiomyocyte-like cells by the introduction of three transcription factors: Gata4, Mef2c and Tbx5 (collectively referred to here as GMT). Resident cardiac fibroblasts can be converted in vivo into induced cardiomyocyte-like cells (iCMs) that closely resemble endogenous cardiomyocytes and electrically integrate with the host myocardium. In contrast, in vitro reprogramming yields many partially reprogrammed iCMs, with a few that reprogram fully into contracting myocytes (~3 out of 10,000 GMT-transduced cells). iCMs can be observed as early as 3 d after viral infection, and they continue to mature over 2 months before beating is observed. Despite the success of multiple groups, the inefficiency of in vitro reprogramming has made it challenging for others. However, given the advantages of in vitro iCMs for performing mechanistic studies and, if refined, for testing drugs or small molecules for personalized medicine and modeling cardiac disease in a dish, it is important to standardize the protocol to improve reproducibility and enhance the technology further. Here we describe a detailed step-by-step protocol for in vitro cardiac reprogramming using retroviruses encoding GMT.